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Boronate functionalized magnetic nanoparticles and off-line hyphenation with capillary electrophoresis for specific extraction and analysis of biomolecules containing *cis*-diols

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ABSTRACT

In recent years, functionalized magnetic nanoparticles (MNPs) have drawn continuously increasing attention due to their great potential for capturing biological molecules or species. However, functionalized MNPs as nanoextraction probes and the coupling with a separation platform for chemical analysis have not extensively investigated yet. In this study, boronate functionalized MNPs were synthesized and employed as extracting probes to capture and enrich *cis*-diol-containing biomolecules, and an off-line coupling method of the MNPs-based extraction with capillary electrophoresis (CE) was established by using pH junction, an on-line preconcentration technique in CE, as a bridge for the coupling. The prepared MNPs exhibited specific selectivity and sufficient capacity. The pH junction compressed a large injected sample volume into a much narrower sample zone and therefore significantly improved the detection sensitivity, solving the sensitivity mismatch between the MNPs-based extraction and CE. Experimental conditions for the pH junction and the desorption were optimized. Under the optimized conditions, the sensitivity was enhanced by 42-fold as compared with regular CE. N,N-dimethylformamide was found to be an effective desorption promoter, which reduced the desorption time to a few minutes. With the established method, riboflavin in a human urine sample was determined.

1. Introduction

Sampling/sample preparation is an important step in analytical approaches. Due to their peerless advantages especially the large surface-to-volume ratio, nanomaterials have been successfully applied as sample preparation support in many analysis tasks [1–5]. Among a variety of nanomaterials, magnetic nanoparticle (MNP) is a very useful one due to its easy manipulation in magnetic separation. Recently, functionalized MNPs as solid-phase extracting agents have increasingly obtained great attentions, particularly for the extraction of biological molecules or species [6–18]. Functionalized MNPs have exhibited significant strengths in three application areas: (1) integrated micro-total-analysis-system (μTAS) [7], (2) in vivo analysis especially single-cell analysis [8–10], and (3) combined with mass spectrometry (MS) for proteomic analysis [11–18]. However, few of these MNP-based extraction studies involve combination with a separation platform. Capillary electrophoresis (CE), as an important microscale separation tool for the analysis of biomolecules, features with high efficiency, high speed and low sample/reagent consumption. It is therefore of great interest to couple MNPs-based extraction with CE. However, such a coupling is challenging, since CE is generally associated with poor detection sensitivity due to the limited light-path while the amount of the analyte extracted by a small amount of MNPs is limited.

In this study, we present the use of boronate functionalized MNPs for specific extracting *cis*-diol-containing biomolecules and a strategy for its combination with CE. Poly-3-aminophenylboronic acid (poly-APBA) coated Fe_3O_4/SiO_2 core/shell magnetite nanoparticles were synthesized as the extraction probes. The extraction is based on covalent bond formation between the boronic acid and 1,2- and 1,3-*cis*-diols at a basic pH condition and reversible release of the *cis*-diol-containing compounds when changing the pH to an acidic condition. The prepared MNPs were characterized in terms of particles size, extraction specificity, extraction capacity and extraction equilibrium. To overcome the sensitivity mismatch issue, pH junction [19,20], an on-line preconcentration technique in CE, was proposed as a bridge to couple the MNPs-based extraction with CE. The pH junction step permits injection of a large sample volume into the capillary and focuses it into a much narrower zone, providing notably improved detection sensitivity. The focusing principle relies on differential electrophoretic velocity induced by significantly different pH values of the background electrolyte (BGE) and the sample zone, which matches well with the pH-controlled capture/release mechanism of the boronate

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affinity extraction. Experimental conditions were optimized for the pH junction and the desorption procedure. Riboflavin (vitamin B_2), a 1,3-*cis*-diol-containing compound that plays significant roles in maintaining the health of humans and animals, was then used as a main analyte. The established method was finally applied to the extraction and determination of riboflavin in human urine sample.

2. Experimental

2.1. Reagents

Tetraethoxysilane (TEOS) was purchased from Sigma (St. Louis, MO, USA). 3-Aminophenylboronic acid (3-APBA) monohydrate and riboflavin were obtained from Alfa Aesar (Tianjin, China). Other chemicals were obtained from normal commercial sources. All chemicals were of analytical reagent grade or higher. Water was purified with a Milli-Q Advantage A10 System (Millipore, Milford, MA, USA), and was used to prepare all solutions.

2.2. Preparation of poly-APBA coated magnetic nanoparticles

The synthesis procedure is comprised of three steps: (1) synthesis of amino functionalized magnetic nanoparticles, (2) preparation of silica shell, and (3) encapsulation with poly-APBA polymer. The amino functionalized MNPs were synthesized according to a previously reported method [21]. Briefly, 1.0 g ferric trichloride hexahydrate, 6.5 g 1,6-hexanediamine and 2.0 g anhydrous sodium acetate were mixed with 30 mL glycol in a PTFE-lined autoclave and reacted at 198 °C for 6 h. The resulting magnetic nanoparticles were rinsed with water and ethanol for 3 times each, and then dried at 50 °C. To cover the amino functionalized MNPs with a silica shell, 0.9 mL TEOS and 5 mL ~8.5% ammonium hydroxide were added in 150 mL ethanol, and the mixture was left to react at room temperature for 20 min. Then 250 mg nanoparticles were added and the reaction was left for another 6 min. The resulting SiO_2/Fe_3O_4 nanoparticles were collected by a magnet at the wall and washed 3 times with ethanol and dried at 50 °C. To make a poly-APBA shell on the SiO_2/Fe_3O_4 nanoparticles, 80 mg SiO_2/Fe_3O_4 was mixed with 4 mL of 80 mM APBA and 4 mL of 100 mM ammonium persulfate and reacted at room temperature for 2 h. Finally, the poly-APBA/SiO_2/Fe_3O_4 nanoparticles were washed 3 times with ethanol, dried at 50° C, and then stored for further use.

2.3. Instruments

All CE separations were performed on a laboratory-rebuilt system with a high-voltage power supply and a laser-induced fluorescence (LIF) detector dissembled from a Unimicro TriSep-2100 pressurized capillary eletrochromatography (pCEC) system (Pleasanton, CA, USA). A bare fused-silica capillary of 70 cm length (50 cm effective length) × 75 μm I.D. × 375 μm O.D. from Yongnian Optical Fiber Factory (Hebei, China) was used as the separation column. Except the inlet and outlet ends, the main length of the capillary was placed in a cardboard box, the temperature within which was controlled around the ambient temperature by forced air delivered by an electric fan. The samples were injected by gravity through elevating the inlet end 30 cm or 10 cm higher relative to the outlet end for a certain time. Transmission electron microscopic (TEM) analyses were performed on a JEM-100 s system (JEOL, Tokyo, Japan) with an acceleration voltage of 80 kV. Samples for the TEM analyses were prepared by drop-wise applying water-dispersed NMPs onto copper grids and then drying naturally. IR spectra were taken on a Nicolet 6700 Fourier transform infrared (FTIR) spectrometer (Thermo Fisher, Franklin, MA, USA). A 1200 HPLC-diode array detection (DAD) system (Agilent Technologies, Waldbronn, Germany) was employed for the verification of the extraction specificity. The HPLC system was controlled by a LC 3D ChemStation (version B.02.01-SR1). A Zorbax Eclipse XDB-C8 analytical column (150 mm × 4.6 mm I.D., 5 μm particle size) from Agilent was used for the separation. The HPLC conditions were as follows: column temperature, 20 °C; mobile phase, methanol/50 mM acetic acid (60:40, v/v); flow rate, 0.5 mL/min; volume injected: 10 μL; and DAD wavelength, 275 nm. Fluorescence measurements were carried out on a RF-5301PC spectroflurophotometer (Shimazu, Kyoto, Japan). UV absorbance measurements were implemented on an UV-3600 spectrometer (Shimazu).

2.4. Measurement of extraction capacity

Direct UV absorbance and fluorescence measurements were carried out to determine the extraction capacity for catechol and riboflavin, respectively. The UV absorbance at 275 nm of a series of standard catechol solutions and the fluorescence intensity at 520 nm (excited at 450 nm) of a series of standard riboflavin solutions were measured to establish calibration curves. A certain amount of MNPs was added into 1 mL of 1 mg/mL catechol or 4 mL of 1 mg/mL riboflavin dissolved in 10 mM sodium carbonate buffer (pH 10.0). The mixture was dispersed by ultrasonication for 30 s and shaken on a rotator for 2 h. Then the MNPs were attracted to the tube wall using a magnet. The collected MNPs were washed for several times with totally 1 mL of 10 mM sodium carbonate buffer (pH 10.0). Then the extracted compounds were desorbed with 10 mM acetic acid of the same volume as the original samples and shaken on a rotator for 1 h. Finally, the MNPs were collected at the tube wall using a magnet and the eluent was transferred into a colorimetric cuvette for absorbance or fluorescence measurement. The extracted amounts were calculated according to the calibration curves, which were considered as the saturated capacity of the MNPs for test compounds under measurement.

2.5. Measurement of extraction equilibrium

The extraction equilibrium was investigated using indirect fluorescence measurement with riboflavin as the test compound. The fluorescence intensities at 520 nm (excited at 450 nm) of a series of standard solutions of riboflavin were measured to establish a calibration curve. 10 mg of MNPs was mixed with 4 ml of 0.57 μg/mL riboflavin solution. The mixture was dispersed by ultrasonication for 30 s and shaken on a rotator for a certain time. Then the MNPs were collected at the tube wall using a magnet and the supernatant was transferred into a colorimetric cuvette for fluorescence measurement under identical conditions. The riboflavin concentration in the supernatant was calculated by the calibration curve. The difference between the original concentration and the concentration found in the supernatant was used to calculate the amount of riboflavin extracted by the boronate functionalized MNPs at a certain extraction time.

2.6. CE conditions

At the beginning of each day, the capillary was rinsed with 1 M NaOH for 30 min followed with the BGE for 1 h. Between runs, the capillary was conditioned with 0.1 M NaOH for 3 min and the BGE for 6 min. A 20 mM sodium tetraborate buffer, pH 10.0, was chosen as the BGE. For normal CE, the riboflavin samples were prepared by diluting the stock riboflavin solution (0.1 mg/mL) with the BGE to get a certain concentration. While for pH junction, the stock solution was diluted with 20 mM phosphate buffer of certain pH to reach a certain concentration. The separation voltage was 15 kV. The excitation wavelength for the LIF detection was 473 nm while the detection wavelength was 520 nm.